



Asian Journal of Plant Sciences

ISSN 1682-3974

science
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Research Article

Chemical and Nutritional Compositions and *in vitro* Digestibility of Brown and Green Seaweed in Southern Oman

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Abstract

Background and Objective: Seaweeds have over the last century been identified as a major source of nutritional and medicinal active agents. In this study, brown alga *Nizamuddinina zanardinii* and green alga *Ulva fasciata* were evaluated for their chemical compositions. **Materials and Methods:** *Nizamuddinina zanardinii* and *Ulva fasciata* were collected, washed vigorously and dried in shade (the temperature around 30°C) for 2-5 days. After that, chemical and nutritional compositions and *in vitro* digestibility were assessed. One-way ANOVA was used to compare the chemical composition, minerals and *in vitro* digestibility of the samples. Multiple comparisons among the samples were performed using a Tukey HSD *post hoc* test. **Results:** The 2 most dominant components in both seaweeds were total carbohydrates (51.31-65.05%, dry weight) and ash (16.34-24.22%, dry weight), however their crude lipid (0.08-0.24%, dry weight) and fibre (5.23-5.45%, dry weight) contents were low. Protein content (13.94%, dry weight) of *Ulva fasciata* was higher than that of *N. zanardinii* (3.86%, dry weight). Atomic spectrophotometry of the minerals has revealed that *U. fasciata* contained greater concentrations of macro-minerals (K, Na, Ca, Mg) but a lower amount of trace elements (Fe, Zn) than *N. zanardinii*. *In vitro* digestibility assays of total dry matter in these seaweeds showed high digestibility (98.37-99.05%, dry weight) whereas protein digestibility in *U. fasciata* (90.46%, dry weight) was higher than in *N. zanardinii* (85.30%, dry weight). **Conclusion:** The findings show that those seaweeds have a high nutritional benefit and should be used as ideal human and animal dietary supplements.

Key words: Chemical composition, digestibility, mineral composition, seaweeds, nutritional benefit

Citation: Al-Souti, A., W. Gallardo and M. Claereboudt, 2022. Chemical and nutritional compositions and *in vitro* digestibility of brown and green seaweed in Southern Oman. Asian J. Plant Sci., 21: 353-359.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Seaweed is the primary marine plants and algae biomass in many coastal regions of the world that includes a larger number of photosynthetic marine eukaryotic species¹. They are known to have rich sources of polysaccharides, secondary metabolites, dietary fibre, vitamins, fatty acids and minerals²⁻⁵. They have some bioactive compounds that are a major resource for drug development⁶. Moreover, in the food industry marine seaweeds are used as bases of phycocolloids, thickening and gelling factors⁷. Their areas are well rich source of antioxidant components^{6,8,9} and it has been reported that brown seaweed comparatively has higher antioxidant potential in comparison to green and red seaweeds⁹.

To date, the nutritional compositions of many types of seaweed have to be established, which are determined primarily by analyzing their chemical composition. In particular, seaweeds have small amounts of lipids and higher levels of dietary carbohydrates and fibres^{10,11}. Nevertheless, the chemical profile of aquatic seaweeds differs based on environmental conditions such as surface temperature, substrate, salinity, light, the abundance of minerals, geographic location and seasons^{12,13}. Digestion of proteins in the seaweeds by proteolytic enzymes such as pepsin, trypsin, pancreatin, pronase, chymotrypsin has been stated^{14,15}. Conversely, the *in vitro* and *in vivo* digestibility study of seaweeds in the Arabian Sea and the Gulf of Oman is very limited.

The coastlines of Oman nearby the Arabian Sea are extremely stressed, but there are some benefits. During summer, a Southwest monsoon (called khareef) is dominated the coastal oceanography of the southern Oman shoreline. Strong Southwesterly winds move horizontally to the shoreline from June-September, causing a heavy coastal upwelling between July and August, followed by maximum wave heights¹⁶. Around June and September, cold, nutrient-rich and turbid waters replace warm oligotrophic coastal waters. The outcome is an upwelling environment that is classified among the world's 5 most extreme upwelling areas^{17,18}. The prevailing conditions during the monsoon allow the dense growth of the kelps *Sargassopsis zanardinii* (Schiff.), *Nizamuddiniana zanardinii*, *Ulva fasciata* and *Eckloniadiata*^{19,20}. However, brown seaweed, *Nizamuddiniana zanardinii* and green seaweed, *Ulva fasciata*, are the most dominated seaweeds in Southern Oman. Owing to the lack of knowledge on their nutritional composition and digestibility, these seaweed products in Oman are currently not used for any nutritional use. In addition, research on nutritional compositions of *Nizamuddiniana zanardinii* and *Ulva fasciata* from the area of Dhofar are very limited.

The objectives of this research was therefore to investigate and determine the chemical composition, minerals and *in vitro* digestibility of brown seaweed, *Nizamuddiniana zanardinii* and green seaweed, *Ulva fasciata*, in Southern Oman collected from November-December, 2018.

MATERIALS AND METHODS

Chemicals: Chemicals were purchased from Oman Lab Scientific and Laboratory and Sigma-Aldrich Co., Oman. They were classified analytically and extra clear.

Seaweed preparation: The brown alga, *Nizamuddiniana zanardinii* and green alga, *Ulva fasciata*, were collected from Salalah (latitude 17_0054N and longitude 54_0532E), Dhofar coastal region, south coast of Oman from November-December, 2018. Voucher specimens were deposited at the Department of Marine Science and Fisheries, College of Agricultural and Marine Sciences, Sultan Qaboos University, under the accession numbers *Nizamuddiniana* 123 and *Ulva* 124.

Freshly collected *N. zanardinii* and *U. fasciata* were carefully washed in seawater and taken to the laboratory. After the epiphytes had been washed by washing vigorously in freshwater, the seaweed was dried in shade (the temperature around 30°C) for 2-5 days. After that, the dried seaweeds were powdered to less than 1 mm particles in size and then stored in wrapped plastic bags at -20°C for lab examination.

Assessment of proximate composition of the samples

Ash and fibre contents: The ash content of seaweeds was quantified gravimetrically according to the procedure of Carneiro *et al.*²¹. It was measured by burning 1 g of samples overnight in a muffle furnace in weighed silica crucibles (GALLEN KAMP, size 2, UK) at 600°C. The ash content was expressed as a percent of dry weight (DW). The fiber content of seaweeds was measured using the enzymatic gravimetric process²¹.

Estimation of total protein from seaweeds: The Kjeldahl method was used to determine the total protein content (Kjeltec Analyzer Unit 2300, Sweden), which includes 3 steps: Digestion, distillation and titration. Data reported as a percentage of Dry Weight (DW).

Estimation of crude lipid from seaweeds: Determination of the crude lipid percentage was done by the Soxhlet method and by using petroleum ether solvent²². The crude lipid was calculated gravimetrically following overnight preservation of the extract at 80°C.

Moisture content of seaweeds: The moisture content of the seaweeds was assessed by drying the fresh seaweeds following the method of Seedeve *et al.*²³. The result was a proportion of percentage.

Assessment of total carbohydrate content of seaweeds: Assessment of total carbohydrate was conducted by phenol-sulphuric acid procedure⁵. The total carbohydrate content of seaweeds was assessed based on the standard glucose curve and the results were expressed as percentages of dry weight.

Assessment of mineral contents: Mineral contents were quantified using atomic absorption spectrophotometry (Thermo Fisher Scientific, Tokyo, Japan)⁵. The assessed minerals were: Potassium, sodium, calcium, magnesium, copper, iron and zinc. Measurements were obtained as mg/100 g dry weight.

In vitro digestibility of seaweeds: *In vitro* digestibility of *N. Zanardinii* and *U. fasciata* was measured using a single-enzyme pepsin-digestible nitrogen (N) assay in each sample²¹. This was done in 0.075 N HCl using 0.2% pepsin (activity 1:10,000). In this experiment, 0.5 g samples were incubated for 16 hrs in a 150 mL solution of pepsin at 45°C. The end-over-end style agitator held twelve 224 mL brown screw cap bottles and maintained temperature in a rotating air-drying oven. All samples were subjected to proximate composition analysis²¹ methods for dry matter and crude protein analysis.

Statistical analysis: One-way ANOVA was used to compare the chemical composition, minerals and *in vitro* digestibility of *N. zanardinii* and *U. fasciata*. Multiple comparisons among the samples were performed using a Tukey HSD *post hoc* test. Chemical composition, minerals and *in vitro* digestibility effects were considered significant at $p < 0.05$. The above statistical analysis was performed using the R statistical package²⁴.

RESULTS AND DISCUSSION

Proximate composition of *N. zanardinii* and *U. fasciata*:

The proximate composition of the *N. zanardinii* and *U. fasciata* obtained from the coastal waters of Salalah is tabulated in Table 1. In general, the amount of ash in the seaweeds is known to be high (ranging from 8-40% dry weight)²⁵ that is indicative of high levels of various minerals in seaweeds²⁶. The ash value in *U. fasciata* ($24.22 \pm 0.02\%$ DW) was significantly higher than *N. zanardinii* ($16.34 \pm 0.11\%$ DW) ($p < 0.05$). The

ash content determined for *U. fasciata* was higher than reported by Rohani-Ghadikolaei *et al.*²⁷ ($12.4 \pm 1.05\%$ DW). On the other hand, the value of ash content in *N. zanardinii* agreed with the finding of Mohammadi *et al.*²⁸ ($15.84 \pm 1.26\%$ DW). The total fiber content of *N. zanardinii* ($5.23 \pm 0.55\%$ DW) and *U. fasciata* ($5.45 \pm 0.33\%$ DW) indicated no significant different ($p < 0.05$). *Nizamuddiniana zanardinii* and *U. fasciata* have higher fibre than *Ascophyllum nodosum*, *Eucheumaisiforme*, *Caulerpa racemosa* and *Codium isthmocladum*, on the other hand, the fibre was lower in these seaweeds when compared to *Sargassum filipendula* and *Padina gymnospora*²⁹. In general, the total fibre content was low in both seaweeds. Dawczynski *et al.*³⁰ stated that fibre promotes growth, protects the healthy digestive system and high seaweed fibre intake lowers colon cancer risk.

The protein content of green seaweed (*U. fasciata*) ($13.92 \pm 0.05\%$ DW) was significantly higher ($p < 0.05$) compared to the brown seaweed (*N. zanardinii*) ($3.86 \pm 0.16\%$ DW). The amount of protein in brown seaweed (*N. zanardinii*) and green seaweed (*U. fasciata*) are within the ranges of Burtin³¹, who recorded relatively lower protein content in brown seaweed (ranging from 3-15% DW) relative to green and red seaweed (ranging from 10-30%). A similar range of protein content in seaweeds was also reported by Chakraborty and Santra³² and Manivannan *et al.*³³. The amount of protein measured in *U. fasciata* in the present study was more than previously for other green species of the seaweed, *U. lactuca* ($7.06 \pm 0.06\%$ DW)¹¹. Whereas, the amount of protein in *N. zanardinii* in the present study agreed with Mohammadi *et al.*²⁸ ($3.18 \pm 0.05\%$ DW). Variations in the protein content of seaweeds can occur due to season, species and environmental conditions³⁰.

The lipid level of seaweeds is generally low (1-5% DW)^{31,34}. The lipid values were not significantly different between the two seaweeds ($p < 0.05$). The levels of lipid detected in the present study for *N. zanardinii* ($0.24 \pm 0.17\%$ DW) was similar to previously reported for other brown seaweed species, specifically: *Hormophysatriquetra* (0.19% DW) and *Sargassum wightii* (0.21% DW)⁹. The crude lipid content of *U. fasciata*

Table 1: Proximate composition (dry weight %) of the *N. zanardinii* and *U. fasciata*

Composition (%)	<i>Nizamuddiniana zanardinii</i>	<i>Ulva fasciata</i>
Ash content	16.34 ± 0.11^b	24.22 ± 0.02^a
Crude fiber content	5.23 ± 0.55^a	5.45 ± 0.33^a
Crude protein content	3.86 ± 0.16^b	13.92 ± 0.05^a
Crude lipid content	0.24 ± 0.17^a	0.08 ± 0.37^a
Moisture content	8.71 ± 0.64^a	8.22 ± 0.52^a
Total carbohydrate content	65.05 ± 0.72^a	51.31 ± 0.65^b

Results expressed as Mean \pm SD (n = 3), different superscript letters in the same row indicate significant differences ($p < 0.05$)

Table 2: Mineral composition (mg/100 g dry weight) determined by atomic absorption spectrophotometry in *N. zanardinii* and *U. fasciata*

Mineral	<i>Nizamuddinina zanardinii</i>	<i>Ulva fasciata</i>
K	5500±1.61 ^b	6810±1.05 ^a
Na	1126±1.38 ^b	1690±1.74 ^a
Ca	829±0.99 ^b	1360±1.82 ^a
Mg	1125±1.37 ^b	1216±1.56 ^a
Cu	4.7±0.82 ^b	9.8±0.46 ^a
Fe	65±0.98 ^a	30±0.83 ^b
Zn	5.8±0.63 ^a	3.2±0.55 ^b

Results expressed as Mean±SD (n = 3), different superscript letters in the same row indicate significant differences (p<0.05)

Table 3: *In vitro* digestibility of dry matter and protein (dry weight %) of *N. zanardinii* and *U. fasciata*

Seaweeds	<i>In vitro</i> digestibility of dry matter (%)	<i>In vitro</i> digestibility of protein (%)
<i>N. zanardinii</i>	98.37±0.72 ^a	85.30±1.06 ^b
<i>U. fasciata</i>	99.05±1.21 ^a	90.46±1.15 ^a

Results expressed as Mean±SD (n = 3), different superscript letters in the same column indicate significant differences (p<0.05)

(0.08±0.37% DW) was significantly lower than that of *U. lactuca* (1.64±0.10% DW)¹⁷. The moisture content determination is among the most basic and scientific methods to be carried out. Moisture is a consistency element in storing some items and influences the durability of foodstuffs⁵. Moisture is often seen as a consistency element and is sometimes defined in normal composition³⁵. The moisture content of *N. zanardinii* and *U. fasciata* was observed as 8.71±0.64 and 8.22±0.52%, respectively.

Carbohydrates are needed for important energy processes such as respiration⁹. Fucoidan, alginate, mannitol, laminaran and cellulose are the common carbohydrates to be found in brown and green seaweeds³⁰. There was a significant difference between total carbohydrates in the seaweeds (p<0.05). These concentrations are higher than those reported by MacArtain *et al.*³⁶ for *Ascophyllum nodosum* (13.1%), *Undaria pinnatifida* (4.6%), *Laminaria digitata* (9.9%) and *Himanthalia elongata* (15%). In addition, they are higher than those found by Wong and Cheung¹¹ for *U. lactuca* (14.6%), Syad *et al.*⁵ for *S. wightii* (0.0095%) and Mohammadi *et al.*²⁸ for *Colpomenia sinuosa* (11.3%). The large variability in carbohydrate content found in various organisms may be attributed to environmental factors such as salinity, temperature and sunlight strength³⁷. In addition, the content of carbohydrates is affected by biomass that reveals the relation between seaweed growth and carbohydrate content¹².

Minerals composition: Seaweeds can be an excellent minerals source and trace elements³⁷. The cell wall polysaccharides and seaweed proteins comprise sulphate, anionic carboxyl and phosphate groups that work as binding for the retention of metals³⁸. The mineral composition of *N. zanardinii* and

U. fasciata are tabulated in Table 2. The green seaweed *U. fasciata* contains significantly (p<0.05) higher levels of potassium, sodium, calcium, magnesium, copper compared to that found in the brown seaweed *N. zanardinii*, except for iron and zinc. The findings reveal that *U. fasciata* has high potassium content (K) (6810±1.05 mg/100 g) and sodium (Na) (1690±1.74 mg/100 g DW) (Table 2). Sodium and potassium help enhance brain functions and perform an important part in the brain's electrical conductivity⁵.

Many of the seaweeds tested in this sample displayed elevated concentrations of potassium, sodium, calcium, magnesium and iron but small quantities of copper and zinc, close to values recorded by Chesalin *et al.*³⁹, although comparatively higher than those stated by Rupérez²⁵ and Rohani-Ghadikolaei *et al.*²⁷. Among all the 7 minerals analyzed, potassium was found to have the highest concentration (5500-6810 mg/100 g DW), but this was comparatively lesser than those seaweeds recorded by Matanjun *et al.*⁴⁰ (8371.2-13155.2 mg/100 g DW).

Seaweeds can specifically accumulate minerals from nearby seawater in their thalli⁴¹. Their mineral content and distribution are also species and location-specific. Edible brown and green seaweed should be consumed as a dietary aid to better achieve the required regular intake of important minerals and trace elements²⁵. The existing data revealed that the seaweed obtained from the Salalah region of the Arab Sea generally contained higher levels of all the minerals analyzed than other vegetables such as lettuce (*Lactuca sativa*) and spinach (*Spinacia oleracea*)⁴². Hence, the present data reveal that *N. zanardinii* and *U. fasciata* may serve as major mineral sources that are vital for human diets.

***In vitro* digestibility of *N. zanardinii* and *U. fasciata*:** The *in vitro* digestibility of *N. zanardinii* and *U. fasciata* was achieved via pepsin-digestible nitrogen (N) single-enzyme assay and the results are illustrated in Table 3. The *in vitro* digestibility of dry matter in these seaweeds (98.37-99.05% DW) are very high and not significantly different (p<0.05) which could be similar to the observation of Santizo *et al.*⁴³. On the other hand, the *in vitro* digestibility of protein in *U. fasciata* (90.46% DW) was significantly different (p<0.05) than *N. zanardinii* (85.30% DW). The *in vitro* digestibility of protein in *U. fasciata* was higher than the other green seaweed *U. lactuca* reported in Wong and Cheung⁴⁴ work, 85.7±1.90%. However, it was comparable to *Ulva* sp. 89.4±2.6%⁴⁵, when the proteins were hydrolysable by trypsin and chymotrypsin.

Fleurence⁴⁶ stated that the ingestion of seaweed proteins *in vitro* depended on the species and seasonal fluctuations in the content of anti-nutritional factors, such as phenolic

residues (e.g., xylans and cellulose) and polysaccharides^{46,47}. The production of protein concentrates from marine algae will be affected by the significant number of hydrophobic or neutral polysaccharides and phytic acid in the cell wall⁴⁸⁻⁵⁰. The tendency of phenolic compounds to develop insoluble protein interacts with the usage of protein intake and hence its nutritious benefit is decreased⁴⁴. Moreover, various *Enteromorpha* sp. solvent extracts (*E. compressa*, *E. linza* and *E. tubulosa*) have good phenol content⁵¹.

CONCLUSION

The brown *N. zanardinii* and green *U. fasciata* seaweeds examined in this study were found to have a considerable amount of chemical and nutritional compositions. However, the chemical and mineral compositions and *in vitro* digestibility of green seaweed *U. fasciata* reveal that this seaweed has a considerable amount of dietary content of ash, protein, minerals and *in vitro* digestibility and low fibre and lipid contents. Concerning these findings, *N. zanardinii* and *U. Fasciata* is healthy food sources for humans and livestock and must be further confirmed through physiological research. However, even further studies (e.g., compositions of amino acids and fatty acids) are critical to enhancing the understanding of the nutritional value of these species, which other people traditionally consume as a vegetable. These results emphasize the feasibility of using seaweeds as new chemical and nutritional sources for human nutrition and industrial food processing.

SIGNIFICANCE STATEMENT

"This study discovered the amount of chemical and nutritional compositions of *N. zanardinii* and green *U. fasciata* seaweeds. This study will help the researchers to expose the sources of seaweeds for human nutrition and industrial food processing in underdeveloped and developing countries".

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